

SYNTHESIS OF NOVEL QUORUM SENSING MOLECULES

By

Lacie Marie Smith

A paper submitted in partial fulfillment of the requirements of the Honors Program in the Department of Chemistry and Biochemistry.

Approved By:

Examining Committee:

\_\_\_\_\_  
Dr. Sridhar Varadarajan, PhD  
Faculty Supervisor

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

\_\_\_\_\_  
Dr. James Reeves, PhD  
Department Chair

\_\_\_\_\_  
Honors Council Representative

\_\_\_\_\_  
Director of the Honors Scholars Program

The University of North Carolina at Wilmington

Wilmington, North Carolina

December 2005

## Table of Contents

Acknowledgments.....	iii
Abstract.....	iv
Introduction.....	1
Design of Molecules.....	3
Experimental.....	4
Results and Discussion.....	10
References.....	14

## ACKNOWLEDGEMENTS

I would like to thank Dr. Sridhar Varadarajan for his time and dedication that he has devoted to this project. I would like to thank the members of my committee, Dr. Pamela Seaton, Dr. Stephen Kinsey, and Dr. Jeffery Wright for their support and guidance, which has been greatly appreciated. This project would not have been done without the prior interest of Dr. Jeffery Wright. I am thankful for his concentration in the subject of quorum sensing. Through Dr. Wright and Dr. Vardarajan's knowledge of quorum sensing I learned information that I never thought I would be interested in. There mentoring has made me a more respected and determined scientist and for that I am grateful. I would also like to thank Dr. Everett Pesci from the Brody School of Medicine at East Carolina University for taking the time to perform the bioassays on all my compounds. Finally, I would like thank the Department of Chemistry and Biochemistry for allowing up coming scientists like me to participate in such research projects in order to become superior chemists.

**Abstract**

Quorum sensing is the phenomenon of communication between bacterial cells. Bacteria communicate with each other through signaling molecules. When a “quorum” is reached the signaling molecules induce virulence factors which allow the bacteria to work in concert to harm the host. This project describes the synthesis of novel quorum sensing molecules that can interfere with bacterial communication. These new molecules are acyl homoserine lactones, similar in structure to that of N-3-(4-hydroxyphenyl)propanoyl homoserine lactone, which had been identified earlier to be a good signaling molecule. These molecules have been made to determine the structural features of N-3-(4-hydroxyphenyl)propanoyl homoserine lactone that are important for its signaling properties. There were four molecules made during this project and the details of their synthesis are described. In all four molecules an acyl component containing a phenyl ring is connected to the homoserine lactone. The molecules differ with respect to the length of the carbon chain tethering the phenyl ring to the homoserine lactone, and with respect to the placement and composition of the substituent on the phenyl ring.

## Introduction

Bacteria are single celled organisms that communicate with one another by a process known as quorum sensing. It is called “quorum sensing” because the phenomenon is initiated when the bacterial population has reached a certain threshold value (quorum) which will allow the bacteria to work in concert.<sup>1</sup> This process is achieved by small signaling molecules, known as autoinducers, which signal the bacteria to produce virulence factors, bioluminescence, etc. The first autoinducers were found in the marine bacterium *Vibrio fischeri* and its close relative *Vibrio harveyi*.<sup>2</sup> Both of these species use acylated homoserine lactone (Figure 1) as the signaling molecules. In *Vibrio fischeri* and *Vibrio harveyi* the use of acylated homoserine lactones regulates the bacterium’s ability to produce bioluminescence.<sup>2</sup>

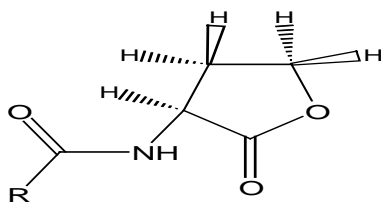


Figure 1: N-acyl homoserine lactone

Gram negative bacteria commonly use acylated homoserine lactones for quorum signaling.<sup>2</sup> Naturally occurring homoserine lactones typically have a saturated hydrocarbon acyl chain tethered to a homoserine lactone. Some examples of naturally occurring homoserine lactones are shown in Figure 2.

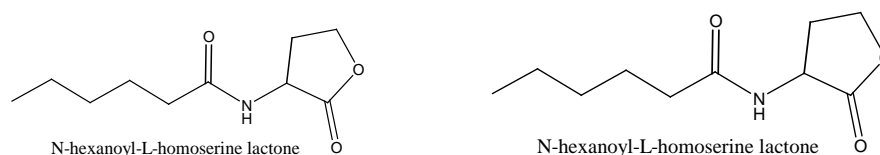


Figure 2

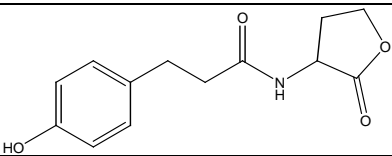
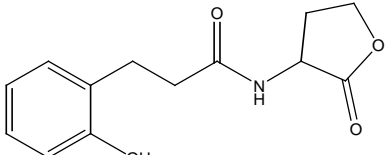
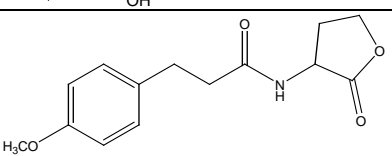
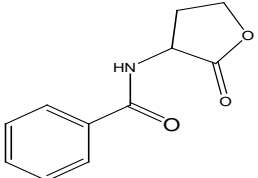
The homoserine lactone is a key component while the acyl side chain can vary from four to fourteen-carbons in length. These side chains can be saturated or can contain functional groups such as carbonyl, hydroxyl, or double bonds.<sup>3</sup> The homoserine lactone's functionality as well as selectivity is dependent on the variations in the acyl side chain.

The main objective of this project is to synthesize novel signaling molecules that will be able to interfere with bacterial communication. These molecules will all contain the homoserine lactone component, but will have variations in the size and composition of the acyl side chain. The study of such molecules could lead to a better design of drugs that can fight bacterial infection and overcome antibiotic resistance.

## Design of molecules

This project was based on a prior project that involved compound **1**<sup>4</sup> (Table 1). Compound **1** was previously synthesized and a bioassay identified this compound as an effective quorum signaling molecule. This compound raised the questions about the structural features of this compound that are important for its quorum signaling properties. To be able to answer these questions, several new molecules were considered with changes in the position of the hydroxy group, with change in the nature of the phenyl substituent (hydroxyl to methoxy), and with changes in the length of the chain linking the homoserine lactone to the phenyl ring. Based on these design considerations, the molecules that were finally made for this project are shown in Table 1.

Table 1: Novel Potential Quorum Signaling Molecules

Number	Structure
<b>1</b>	
<b>2</b>	
<b>3</b>	
<b>4</b>	

## Experimental

### General

All chemicals were obtained from VWR or Aldrich, and were of the highest purity available.  $^1\text{H}$  NMR spectra were obtained in  $\text{CDCl}_3$  or deuterated DMSO using a Bruker 400 MHz spectrometer. Melting points were obtained using a Meltemp melting point apparatus. High pressure hydrogenation was carried out on a Parr Hydrogenator. Filtrations using a buchner funnel were carried out using a Whatman 55 mm filter paper. For moisture sensitive reactions, all glassware were dried in the oven at  $110\text{ }^\circ\text{C}$  overnight, assembled under argon or nitrogen while still hot, and maintained under an argon or nitrogen atmosphere, or fitted with a drying tube, throughout the experiment.

### Synthesis of the N-carbobenzyloxy-L-homoserine lactone (N-CBZ-HSL, 5)

This procedure was modified from a previously described procedure.<sup>4</sup> A solution containing N-carbobenzyloxy-L-methionine (1.7 g, 4.4 mmol), acetic acid (1.3 mL), formic acid (2.64 mL), and an excess of iodomethane (8 mL) was placed in a round bottom flask and allowed to sit in the dark for approximately for 12 hours. The mixture was then concentrated by rotary evaporation. A viscous, yellow-orange oil remained in the flask. This oil was then triturated with 15 mL of anhydrous ether with vigorous stirring. The two layers that formed were then allowed to separate, and the top yellow ether layer was removed with a pipette. The trace amount of ether remaining in the flask was then removed by rotary evaporation. The viscous product left in the flask was dissolved in 5 mL of distilled water, and the pH was adjusted



between 6 and 7 by dropwise addition 1M NaOH, at which point the yellow solution became colorless. The flask was then fitted with a reflux condenser and the solution was heated at 90 °C for 3 hours, with stirring, while maintaining the pH between 6 and 7 by further addition of 1M NaOH. After 3 hours the solution was allowed to cool to room temperature and then the flask was placed in an ice-water bath. White crystals were formed, and after the crystallization was complete, the crystals were filtered through a buchner funnel. The crystals were washed with cold distilled water and then transferred to a round bottom flask and dried under vacuum to yield 0.716 g of **5** (80% yield), mp 126-127 °C; TLC  $R_f$  = 0.66 (ethyl acetate);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): 7.33 (s, 5H), 5.39 (s, 1H, broad), 5.14 (s, 2H), 4.44 (t, 1H), 4.27-4.21 (m, 1H), 2.77 (s, 1H, broad) and 2.26-2.17 (m, 1H);  $^1\text{H NMR}$  (DMSO): 7.79 (d, 1H), 7.35 (s, 5H), 5.04 (s, 2H), 4.43 (q, 1H), 4.30 (t, 1H), 4.19-4.17 (m, 1H), 2.40-2.38 (m, 1H), and 2.18-2.13 (m, 1H).

#### Synthesis of homoserine lactone hydrochloride salt (HSL salt, **6**)

N-CBzHSL (**5**) (2 g, 0.0839 mmoles) was completely dissolved in 95% ethyl alcohol (55 mL) in a hydrogenation Parr jar, and 1 g of 10% Pd/C was added to the solution. The jar was then flushed with  $\text{H}_2$  gas and evacuated twice, and then filled with  $\text{H}_2$  to a pressure of 60 psi, and shaken on the hydrogenator until TLC (100% ethyl acetate) indicated the absence of the starting material. The Pd/C was then filtered off using a buchner funnel and washed thoroughly with ethanol. The filtrate and washings were then transferred to a round bottom flask and 1 M HCl (16.78 mL)

was added. The solution was then concentrated on a rotary evaporator and placed under vacuum to yield 0.0872 g of compound **6** (76.3% yield) as a white crystalline solid.  $^1\text{H}$  NMR (DMSO): 8.8 (s, 3H, broad), 4.46 (t, 1H), 4.35-4.20 (m, 2H), 2.60-2.45 (m, 1H, broad), and 2.28 (quintet, 1H).

#### Synthesis of N-3-(4-hydroxyphenyl) propanoyl HSL (**1**)

This procedure was modified from a previously described project.<sup>4</sup> 3-(4-hydroxyphenyl)propanoic acid N-hydroxysuccinimide ester (100 mg, 3.80 mmol), **5** (100 mg, 4.26 mmol), 10% Pd/C (50 mg), and 30 mL of ethanol were all added to a hydrogenation Parr jar. The jar was flushed with  $\text{H}_2$  gas and evacuated twice, and then filled with  $\text{H}_2$  to a pressure of 60 psi and shaken on the hydrogenator until TLC (100% ethyl acetate) indicated the absence of the starting reactants. TLC showed the formation of two spots more polar than the starting material during the reaction.

The Pd/C was then filtered off using a buchner funnel and washed thoroughly with ethanol. The filtrate and the washings were then transferred to round bottom flask and the concentrated by rotary evaporation. The resulting brown-orange oil was then dried under vacuum. Flash column chromatography (eluent: 50% ethyl acetate: methanol) was then used to purify the product. The white solid residue obtained was then recrystallized from ethyl acetate at  $-20\text{ }^\circ\text{C}$ . The ethyl acetate was then removed and the crystals were dried under vacuum to give 0.0483 g (46% yield) of **1**: mp  $121.1\text{-}126.7\text{ }^\circ\text{C}$ ; TLC:  $R_f = 0.43$  (100% ethyl acetate);  $^1\text{H}$ -NMR (DMSO): 9.13 (s,

1H), 8.34 (d, 1H), 6.97 (d, 2H), 6.63 (d, 2H), 4.51 (q, 1H), 4.32 (t, 1H), 4.21-4.15 (m, 1H), 2.68 (t, 2H), 2.35-2.31 (m, 3H), and 2.08 (quintet, 1H).

### Synthesis of N-3-(2-hydroxyphenyl)propanoyl HSL (2)

To a dry flask equipped with a drying tube, 3-(2-hydroxyphenyl)propanoic acid (0.2 g, 0.012 mmoles), 10 mL of dry CH<sub>2</sub>Cl<sub>2</sub> and SOCl<sub>2</sub> (428 μL, 0.036 mmoles) were added in sequence and the solution was refluxed until TLC (3:2 ethyl acetate: hexane) indicated that a less polar compound (presumably the acid chloride) was formed and no further change in the reaction was taking place (approx. 48 hours). The solution was then concentrated on a rotary evaporator and the resulting viscous oil was placed under vacuum overnight. Dry CH<sub>2</sub>Cl<sub>2</sub> (10 mL) was added to this oil in the flask along with compound **6** (0.181 g, 0.0132 mmoles), and triethylamine (502 μL, 0.036 mmoles), in sequence, and the mixture was refluxed until TLC (3:2 ethyl acetate: hexane) indicated that there was no more acid chloride (least polar spot) present. The solution was then taken up in a separatory funnel, 10 mL of dichloromethane added, and the organic layer was washed with 1M HCl (1 x 10 mL), water (1 x 10 mL), NaHCO<sub>3</sub> (1 x 10 mL), and water (1 x 10 mL). The organic layer was then dried over anhydrous MgSO<sub>4</sub>, the MgSO<sub>4</sub> filtered off, and the filtrate concentrated by rotary evaporation and dried under vacuum to yield 0.126 g of **2** (43.4% yield) as a brown solid: mp 103.1-107.2 °C; TLC R<sub>f</sub> = 0.41 (ethyl acetate); <sup>1</sup>H NMR (DMSO): 9.32 (s, 1H), 8.35 (d, 1H), 7.03 (d, 1H), 6.98 (t, 1H), 6.75 (d, 1H),

6.67 (t, 1H), 4.53 (q, 1H), 4.33 (t, 1H), 4.22-4.17 (m, 1H), 2.72 (t, 2H), 2.37-2.50 (m, 3H) and 2.13-2.08 (m, 1H);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): 8.09 (s, 1H), 7.15-7.06 (m, 2H), 6.91-6.84 (m, 2H), 6.09 (s, 1H, broad), 4.53-4.44 (m, 2H), 4.30-4.24 (m, 1H), 2.94-2.92 (m, 2H), 2.89-2.80 (m, 1H), 2.77-2.67 (m, 2H), and 2.10-1.10 (m, 1H).

### Synthesis of N-3-(4-methoxyphenyl)propanoyl HSL (**3**)

To a dry flask equipped with a drying tube, 3-(4-methoxyphenyl) propanoic acid (0.0528 g, 2.93 mmol), 10 mL of dry  $\text{CH}_2\text{Cl}_2$  and  $\text{SOCl}_2$  (64  $\mu\text{L}$ , 8.79 mmol) were added in sequence and the reaction was performed and worked up as described above for **2**. After the acid chloride was dried under vacuum, dry  $\text{CH}_2\text{Cl}_2$  (10 mL), **6** (0.044 g, 3.23 mmol) and triethylamine (123  $\mu\text{L}$ , 8.79 mmol) were added in sequence, and the reaction carried out and worked up as described for **2** to yield 0.049 g of **3** (64% yield) as a white solid: mp 148.2-156.9  $^\circ\text{C}$ ; TLC  $R_f$  = 0.41 (ethyl acetate);  $^1\text{H NMR}$  (DMSO): 8.36 (d, 1H), 7.10 (d, 2H), 6.81 (d, 2H), 4.52 (q, 1H), 4.32 (t, 1H), 4.20-4.17 (m, 1H), 3.71 (s, 3H), 2.74 (t, 2H), 2.35 (m, 3H), and 2.11-2.06 (m, 1H);  $^1\text{H NMR}$  ( $\text{CDCl}_3$ ): 7.11 (d, 2H), 6.83 (d, 2H), 5.84 (s, 1H, broad), 4.54-4.48 (m, 1H), 4.53 (t, 1H), 4.30-4.24 (m, 1H), 3.80 (s, 3H), 2.93 (t, 2H), 2.87-2.80 (m, 1H), 2.59-2.46 (m, 2H), and 2.08-1.97 (m, 1H).

Synthesis of the N-benzoyl HSL (4)

Dry dichloromethane (4 mL), **6** (0.0337 g, 0.0245 mmol), benzoyl chloride (284  $\mu$ L, 0.0245 mmol), and triethylamine (103  $\mu$ L, 0.0735 mmol) were added, in sequence, to a dry flask equipped with a stir bar, under argon or nitrogen. The reaction was allowed to stir at room temperature until no benzoyl chloride was detected by TLC in 100% ethyl acetate. The solvent and excess triethylamine were then removed by rotary evaporation. The product was then purified by flash column chromatography (eluent: 100% ethyl acetate) to give 33 mg (66.1 % yield) of **4** as a yellow solid: TLC  $R_f$  = 0.48 (ethyl acetate); mp 140.2-142.3  $^{\circ}$ C;  $^1\text{H}$  NMR (DMSO): 8.98 (d, 1H), 7.84 (t, 2H), 7.56 (t, 1H), 7.49 (t, 2H), 4.76 (q, 1H), 4.41 (t, 1H), 4.28-4.25 (m, 1H), 2.49 (m, 1H, broad), and 2.35-2.32 (m, 1H);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ): 7.76 (d, 2H), 7.46 (t, 1H), 7.37 (t, 2H), 7.12 (s, 1H, broad), 4.75-4.68 (m, 1H), 4.46 (td, 1H), 4.32-4.25 (m, 1H), 2.88-2.81 (m, 1H), and 2.29-2.18 (m, 1H).

## Results and Discussion

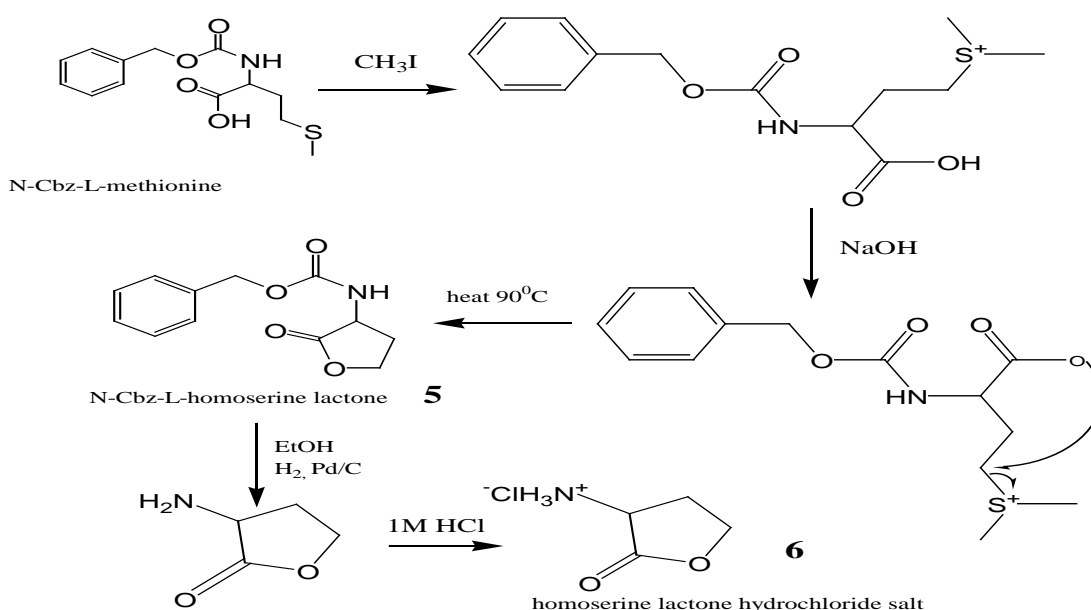
The observation that compound **1** was a quorum signaling molecule prompted questions regarding the structural features of this molecule that were essential for quorum signaling. Also of interest was the question as to whether small changes in **1**, which is an agonist, could result in a molecule that would behave as an antagonist, that is, prevent quorum signaling in bacteria. In order to address these questions, new molecules were designed with systematic variations in the structure, relative to **1**. Since a review of literature indicated that the homoserine lactone component is present in all natural signaling molecules isolated from gram negative bacteria,<sup>1,2</sup> it was decided that modifications would be made in the acyl chain of compound **1**. Modifications in the length of the carbon tether in the acyl chain, the requirement of a H-bond donor on the aromatic ring, and the location of the OH group on the phenyl ring were all considered. Based on these design considerations, the molecules synthesized thus far are shown in Table 1.

The overall synthetic strategy was to combine the various acyl components as the corresponding activated carboxylic acids with the homoserine lactone in the free amine form. Therefore, the homoserine lactone was first synthesized on a large scale, and stored in the form of the hydrochloride salt until required. The free amine was then generated *in situ*, using triethylamine, and condensed with activated carboxylic acids, to yield the desired product.

In order to synthesize the homoserine lactone, N-carbobenzyloxy-L-methionine was S-methylated and then the pH adjusted to between 6 and 7. At this

pH intramolecular nucleophilic attack of carboxylate anion eliminated dimethyl sulfide and resulted in the formation of the lactone ring as shown in Scheme 1. Catalytic hydrogenation of this N-carbobenzyloxy-L-homoserine lactone resulted in the formation of the free amine which was immediately converted to the hydrochloride salt by treatment with 1 M HCl. The overall yield for the formation of the hydrochloride salt was 76 %.

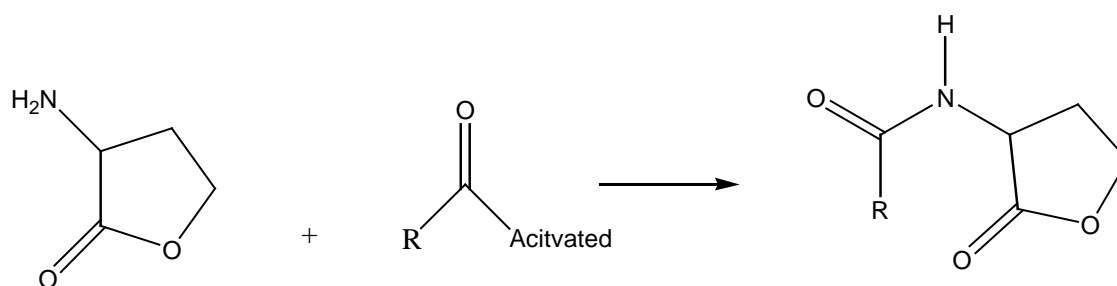
**Scheme 1:** Synthetic scheme of homoserine lactone hydrochloride



The appropriate carboxylic acids were typically activated by conversion into the acid chloride using thionyl chloride. The acid chlorides were used directly in subsequent condensation reactions with the homoserine lactone after removal of solvent and excess thionyl chloride, without further purification. After condensation reactions, the desired products were isolated, purified by flash column

chromatography, and characterized. The general method to make the desired novel quorum signaling molecules is shown in Scheme 2. No attempt was made to optimize the reactions. The percentage yields of the various condensation reactions are listed in Table 2.

Scheme 2: Synthetic scheme for novel quorum signaling molecules



Compound	% Yield
<b>1</b>	46
<b>2</b>	43
<b>3</b>	64
<b>4</b>	66
<b>5</b>	80
<b>6</b>	76

Table 2: Yields of Compounds

The sample of **1** tested earlier contained a small percentage of N-hydroxysuccinimide, (which was formed during the synthesis) as an impurity. In order to verify that this impurity did not interfere with the bioassay the new sample of



**1** was rigorously purified by flash column chromatography and recrystallization in order to obtain a pure sample of **1**. A pure sample of the contaminant N-hydroxysuccinimide was also obtained.

The compounds synthesized in this project, including the contaminant N-hydroxysuccinimide are currently being assayed for their quorum signaling properties in collaboration with Dr. Everett Pesci from the Brody School of Medicine at East Carolina University. Other compounds, with different modifications in the structure of **1**, are also being prepared, and will be subsequently tested. The results from the quorum signaling studies will provide insight into the structural requirements necessary for bacterial signaling in this class of compounds, and perhaps identify compounds that can be used as antagonists of bacterial signaling. Such antagonists could be used to interfere with bacterial communication and fight bacterial infection by new mechanisms, thus circumventing current problems with antibiotic resistance.

**References**

- 1) Bentley W; March J; Quorum sensing and bacterial cross-talk in biotechnology. *Biotechnology* 2004,15: 495-502.
- 2) Bassler Bonnie: How bacteria talk to each other: regulation of gene expression by quorum sensing, *Microbiology* 1999, 2: 582-587.
- 3) Schaefer A.; Hanzelka B.; Parsek M.; Greenberg E.; *Methods of Enzymology*, Vol 305 2000, 288-301.
- 4) Schlobohm, Lauren. Honors Thesis, UNCW Department of Chemistry, May 2004.